Infection of Breast Epithelial Cells With Epstein-Barr Virus Via Cell-to-Cell Contact

Peter Speck, Richard Longnecker

Epstein-Barr virus (EBV), a human herpesvirus present in more than 90% of adults, is a major viral cofactor in certain tumors of lymphoid and epithelial tissues (1). Persistent infection is associated with malignancies and proliferative syndromes typically of lymphoid and epithelial tissues (1), including Burkitt’s lymphoma, Hodgkin’s disease, certain adult T-cell lymphomas, and, in epithelium, nasopharyngeal carcinoma and oral hairy leukoplakia. In vitro, EBV efficiently infects, transforms, and immortalizes B cells, yielding lymphoblastoid cell lines (LCLs).

Several studies have associated EBV with breast cancer. Bonnet et al. (2) detected EBV genomes and gene expression in breast cancer lesions by using polymerase chain reaction (PCR) analysis, Southern hybridization, and immunohistochemistry specific for EBV protein EBNA (i.e., EBV nuclear antigen)-1. Labrecque et al. (3) detected EBV in breast cancers by PCR and in situ hybridization. There are descriptions of EBV-associated lymphomas (4,5) localizing to breast and of bilateral breast cancer developing during the rare chronic active EBV infection syndrome (6).

Recent reports (7,8) have described EBV infection of human carcinoma cells on cocultivation with LCLs by a mechanism requiring cell-to-cell contact. These findings and the reported association with breast cancer prompted us to address the question of whether EBV enters breast epithelium by cell-to-cell contact. We have developed an appropriate reagent: EBV bearing the gene encoding and expressing the protein known as enhanced green fluorescent protein (EGFP) (9,10).

Cells infected by this virus, designated EBfaV-GFP, are readily detected by their green fluorescence (9–11).

Here, we report that cells of human breast cancer epithelial lines T47D and MCF7, which are not infected on direct exposure to cell-free EBfaV-GFP virus, become infected when cocultivated with LCLs derived with and bearing EBfaV-GFP, as shown by expression of EGFP. This finding is consistent with a possible role for EBV in the etiology of breast cancer.

EBfaV-GFP, with EGFP driven by a strong promoter, within a dispensable region of the viral genome is produced as described previously (10). MCF7 and T47D cells (derived from human breast tumors) and Daudi cells (an LCL immortalized by and bearing wild-type

Affiliation of authors: Microbiology–Immunology Department, Northwestern University Medical School, Chicago, IL.

Correspondence to: Richard Longnecker, Ph.D., Microbiology–Immunology Department, Northwestern University Medical School, 622 East Chicago Ave., Chicago, IL 60611 (e-mail: r-longnecker@northwestern.edu).

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EBV) were obtained from the American Type Culture Collection (ATCC), Manassas, VA, and were cultured according to the recommendations of the ATCC. Binding of monoclonal antibody 323/A3 (Lab Vision, Fremont, CA) against epithelial-specific antigen (ESA), abundant on the surface of T47D and MCF7 cells, and Cy5-conjugated goat-anti-mouse secondary antibody (Jackson ImmunoResearch, West Grove, PA) were measured by flow cytometry as described previously (9). Antibody 72A1 (12) against EBV glycoprotein gp350 was used in antibody-blocking experiments. Plasmid pEGFP.N1 (Clontech Laboratories, Inc., Palo Alto, CA), used in control experiments to achieve transient expression of EGFP, was transfected by electroporation into Daudi cells as described previously (10).

Monolayers of T47D or MCF7 cells in 12-well plates, at 50% confluence, were overlaid with equal numbers of GFP57 cells, which is an EGFP-positive lymphoblastoid cell line derived from primary B cells, by the method described previously (10) with the use of EBfaV-GFP virus. In control experiments, monolayers either were overlaid with equal numbers of Daudi cells transiently expressing EGFP (after adjustment for the proportion of cells expressing EGFP) or were exposed to cell-free filtered EBfaV-GFP virus produced as described previously (9) and assayed for infectious titer with the use of Daudi cells. After 24 hours of cocultivation, supernatants were removed, and cell monolayers were washed repeatedly with medium and visually examined with the use of an inverted fluorescence microscope (Leica Microsystems, Wetzlar, Germany). In monolayers cocultivated with GFP57 lymphoblastoid cells, approximately 1%–3% of cells within the monolayer expressed ESA (Fig. 1, panel 2, showing T47D cells), indicating that infection had occurred. In addition, a small number of rounded EGFP-positive cells, with morphology resembling that of LCLs, appeared to be adhering to the top of the monolayer. No EGFP-expressing cells were present within monolayers exposed to cells transiently expressing EGFP (Fig. 1, panel 4), and again a small number of rounded EGFP-positive cells, morphologically resembling LCLs, appeared to be adhering to the top of the monolayer. Similar results were seen with MCF7 cells (data not shown).

The abundant expression of ESA by T47D and MCF7 cells is shown in Fig. 2 (panels 1 and 2). GFP57 and Daudi cells do not express ESA (panels 3 and 4). T47D and MCF7 cells are not infected by direct contact (Fig. 2, panels 6 and 7).
with a 100-μL inoculum of cell-free EBfAV-GFP virus that contained sufficient virus to infect 20% of 10^5 Daudi cells, as measured by the proportion of cells expressing EGFP (Fig. 2, panel 5).

To confirm that EGFP expression was occurring in breast epithelial cells in the monolayer and not in GFP57 cells, two-color flow cytometry was applied with the use of a marker present only on the epithelial cell population. Cells were removed, resuspended, and reacted with antibody 323A3; infected epithelial cells—defined as positive for both ESA and EGFP—were then enumerated. Results (Fig. 2, panels 9 and 10) show that 1.1% and 3.4% of cocultivated MCF7 and T47D cells, respectively, were ESA positive and EGFP positive, confirming infection by cell-to-cell contact. Control experiments, in which cocultivated breast cell monolayers with Daudi cells transiently expressed EGFP (Fig. 2, panels 11 and 12), yielded minimal background (≤0.10%) of dual-positive cells. The same level of background was seen when EGFP-expressing Daudi cells and T47D or MCF7 cells were mixed immediately before flow cytometry, suggesting that the background level did not arise during cocultivation (data not shown).

To address the possibility that LCLs on cultivation yielded free virus that then infected by direct virus-cell contact, cocultivations were repeated with a blocking antibody. Antibody 72A1 against EBV glycoprotein gp350, when included in cocultivations at a range of concentrations (0–40 μg/mL), the highest of which completely abrogates infection of Daudi cells by EBfAV-GFP virus, did not reduce the proportion of T47D or MCF7 cells that became infected (data not shown). We conclude that EBV cell-to-cell infection of these epithelial cells does not require the presence of free virus.

Efficient infection of T47D and MCF7 cells by cell-to-cell contact requires actively growing cells. Repeating cocultivation experiments with the use of completely confluent monolayers yielded cells positive for both ESA and EGFP numbering between 0.2% and 0.3% of total cells, which was two to three times the background level (data not shown).

Previous reports (7,8) have shown that EBV infects epithelial cells by cell-to-cell contact. Our observation is consistent with these findings and extends to breast epithelium the range of tissue types potentially infectible by EBV; it also supports the notion that virus-bearing lymphocytes may serve as virus donors for infection of epithelial cells (8). Cell-to-cell spread by fusion of infected cells with uninfected cells has been documented for other viruses, e.g., herpes simplex virus (13,14), pseudorabies virus (15), human immunodeficiency virus (16), and paramyxoviruses such as measles (17). Although the current studies do not elucidate the mechanism of the cell-to-cell spread of EBV, fusion of infected cells with uninfected cells is a possible explanation for this phenomenon. Alternatively, close cell-to-cell contact could augment the accessibility of virus to recipient cells, possibly with viral attachment and entry via a hypothetical low-affinity receptor molecule or molecules. Examination of the mechanism will be a subject of future experiments. EBV association with epithelial tumors has been difficult to recognize with the apparent inability of EBV virions to efficiently undergo direct entry into epithelial cells, which express little, if any, CD21 (the major receptor for EBV). These observations begin to address the difficulties in understanding a role for EBV in breast cancer etiology by demonstrating EBV entry into breast epithelium by cell-to-cell contact.

REFERENCES


NOTES

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